WELLES

Epithelial Degeneration,
Regeneration & Secretion
In the Mid-Intestine
Of Collembola

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EPITHELIAL DEGENERATION, REGENERATION AND SECRETION IN THE MID-INTESTINE OF COLLEMBOLA

BY

MIRIAM URSULA WELLES, A. B., 1903

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OF Master of Arts.

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EPITHELIAL DEGENERATION, REGENERATION AND SECRETION IN THE MID-INTESTINE OF COLLEMBOLA.

by

MIRIAM U. WELLES.

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I. INTRODUCTION.

This paper deals chiefly with a remarkable process of epithelial degeneration which occurs periodically in the collembolan stomach and has an important physiological significance; and deals secondarily with regeneration and secretion in the stomach of Collembola as compared with those functions in other insects.

The details of this process of degeneration are here described and interpreted for the first time. The only previous references to the subject are by Sommer ('85, p.713), who mentions that he found two different aspects of the epithelium of the mid-intestine of Tomocerus (Macrotoma), and by Fernald ('90, p. 455), who makes a brief reference to the process as observed by him in Anurida maritima.

The work has been done under the supervision of Dr. J. W. Folsom, to whom I am deeply indebted for constant and valuable advice.

II. MATERIAL AND METHODS.

The species of Collembola used in this study were Tomocerus niger, Podura aquatica, Isotoma viridis and Orchesella cincta.

By examining large numbers of Tomocerus, I was able to obtain a sufficiently complete series of stages in the degeneration and regeneration of the stomach epithelium. It was unnecessary to have as complete a series in the case of the other species, since they were found to present essentially the same conditions as Tomocerus,- whence I am led to believe that the phenomena as described here for Tomocerus are the same in all Collembola. Specimens taken at random and sectioned showed striking differences in the condition of the stomach epithelium. In order to ascertain what relation, if any, existed between the condition of the stomach epithelium and that of the external integument, it was necessary to kill the insect with known reference to the time of the external moult. To this end a large number of insects were collected and put, singly, into small glass tubes, with bits of moist, decayed wood, for food, the tubes being closed by cotton plugs. When proper precautions were taken, including the use of sterilized cotton and distilled water, to prevent the growth of fungi, it was found that the insects could be kept alive for three or four weeks, and their habits of feeding, moulting, etc., readily observed.

Collembola moult throughout life, continuing to moult after they are full grown.

This fact, already noticed by Sommer, is of special significance, as indicating that ecdysis is here, at least, something

more than a provision for growth. In Collembola, it does not depend upon temperature or lack of food, and may prove to be an excretory process in these forms, which are unique among insects in having no Malpighian tubes, notwithstanding the statement of Nicolet ('41), Tullberg ('72), and Sommer ('85).

The interval between two successive moults increases with the age of the individual. Very young insects moult as often as every two days, while adult specimens moult at intervals varying from six to eight days. For some time (about twenty-four hours) previous to moulting the insects are inactive, and take no food, but immediately after ecdysis the insect usually devours the cast skin,-- a habit which necessitates almost constant observation in order to determine the time of the moult, before the exuviae have disappeared. The eaten skin, with its numerous chitinous scales, may easily be recognized in the stomach of the insect, and these remains are of use as indicating when the last moult occurred.

The material was studied by means of microtome sections and in dissections, made under the compound or dissecting microscope. Usually, sections of the entire insect were made, but often the stomach was dissected out, and then sectioned. Either method gave excellent results, but the latter is the better, theoretically, as regards fixation, orientation and ease of sectioning. The structural conditions in fixed and stained material were compared critically with those in the living cytoplasm, which was obtained by dissecting out the stomachs of decapitated insects. Both preserved and living cytoplasm agreed precisely in structure, so far as it was possible to observe. The living cytoplasm is alveolated in the same manner as the preserved cytoplasm,—an important fact,

since the transformations in the epithelium of the stomach are largely changes in alveolation, as my figures show.

For killing and fixing the tissues, I used, with excellent results, a saturated solution of mercuric chloride in thirty-five per cent. alcohol, with two percent. of glacial acetic acid. This solution was used at a temperature of about 80° C., and allowed to act for three or four minutes, after which the tissues were left in seventy per cent. alcohol for twenty-four hours in order thoroughly to remove the mercuric chloride. For embedding, the usual paraffin method was employed, using watch glasses, as being most convenient. Serial sections were cut, with a Minot-Zimmermann microtome, from 6 2/3 to 10 micra in thickness, in sagittal and transverse planes.

Dissected material was stained in toto, immediately after washing out the fixing agent, Grenacher's alcoholic borax-carmine being used in this case. The tissues were left in this stain for twenty-four hours, then decolorized in alcohol of seventy per cent. acidulated with a few drops of hydrochloric acid, until a clear, bright red color was obtained.

For staining sections, Ehrlich's acid haematoxylin was used, alone or with safranin or orange G. The tissues were stained in the haematoxylin for about ten minutes, then decolorized in slightly acidulated alcohol, one or two minutes, or until they showed good differentiation. In double-staining, the tissues were treated as above with haematoxylin, and counter-stained in a weak solution of safranin in absolute alcohol, or else a dilute solution of orange G. in ninety-five per cent. alcohol. Both safranin and orange G. are excellent chitin stains. The best results as regards precision

and range of differentiation were obtained with Ehrlich's haematoxylin and orange G.

III. THE ALIMENTARY TRACT. (Pl. 1, Fig. 1).

In Collembola, and Thysanura (especially Campodea and Japyx*) the alimentary tube presents primitively simple conditions, such as in the less primitive insects (Pterygota) are confined to the egg or larva. In Tomocerus, as in all other elongate collembolans, the digestive tube is axial in position, extends directly from the mouth to the anus, and falls into the three distinct regions, namely, fore-intestine (Fig. 1, f. int.), comprising mouth, pharynx and oesophagus, and derived from the ectodermal stomodaeum; mid-intestine (m. int.), from the endodermal mesenteron; and hind-intestine, (h. int.), from the ectodermal proctodaeum. There are no diverticula excepting the salivary glands. The posterior end of the oesophagus folds into the stomach to form a cardiac valve, (car.) and at the posterior end of the stomach a pyloric valve (pyl.) occurs.

The anatomy of the entire digestive tube need not detain us, since it has already been described by Tullberg ('72), Sommer ('85), Fernald ('90), Folsom ('99), and Willem ('00). It is sufficient to say that the wall of the tube consists of a single layer of epithelial cells, which secrete entally a distinct intima (Pl. 2, Fig. 2, in.), and ectally a delicate, hyaline, homogeneous basement membrane, or tunica propria (b.m.). This is surrounded by a single layer of circular muscles (c.m.) which are most strongly developed around the pharynx and rectum. Outside the circular muscles is a single layer

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*Grassi.

of longitudinal muscles (Fig. 1, l.m.) in the case of the stomach; ~~but~~ they being ~~are~~ absent around the oesophagus and rectum.

The mid-intestine, otherwise known as ventriculus, chylific stomach, or stomach*, *[footnote] [this term is in current use, and is often convenient to employ, provided it be understood that the stomach of an insect is entirely different, physiologically, from the stomach of a vertebrate], is the principal division of the alimentary tract. In the adult insect (Tomocerus) the mid-intestine is about two millimeters in length, reaching from the beginning of the mesothorax to the fourth abdominal segment. The average diameter is about one hundred micra. The ventriculus is surrounded, immediately by about ninety circular muscles, and next by about sixteen longitudinal muscles; both kinds of muscle-fibers are distinctly striated. As these muscles undergo no morphological changes in the adult insect, they will seldom be referred to here. I now pass to a consideration of the complex changes undergone by the epithelial cells. These changes are, of course, continuous, but may for convenience of description, be referred to five stages.

IV. DEGENERATION.

Stage I. (Pl. 2, Fig. 2).

The degeneration of the epithelium of the stomach occurs only at each moult, and then occupies but one or two days, at most. At all other times, i.e., between moults, the epithelium is in what I may term a resting condition, since it is characterized by the absence of nuclear division, though the epithelium is of course, active so far as secretion and absorption are concerned.

In the resting condition, there is, as has been said, a single layer of cells; these are now columnar (Fig.2), and ~~hexagonal~~ polygonal in cross-section, (Pl.2, Fig.3); the average length of each cell is about eighteen micra, and the width, four to six micra.

The cytoplasm has, under low magnification, a somewhat granular appearance; under a one-tenth homogeneous immersion-objective, however, a finely alveolar structure appears. This is due to round or oval vacuoles or alveoli (Fig.2, alv.), containing a non-stainable fluid, and separated from one another by a stainable substance which appears in section as a reticulum. Precisely the same appearances are given by the living cytoplasm of these cells. In sectioned and stained material the reticulum frequently presents a suggestion of a granular structure, not sufficiently distinct, however, to be represented in my figures. Around the nucleus, the cytoplasm is less dense than elsewhere, owing to a coarser alveolation (Fig.2). The cytoplasm becomes somewhat denser toward the inner half of the cell, i.e., toward the intima.

Adjoining cells are separated by cell walls(lat.), though these are often indistinct and incomplete, in which case the cells are usually distinguishable from each other only by differences in the density of the cytoplasm and by the positions of the nuclei. The dividing wall is usually most distinct toward the base of the cell. It has not the appearance of a secretion, but rather that of a condensation of the ordinary reticular substance. It stains in the same way as does this inter-vacuolar substance in other parts of the cell, but somewhat more deeply, being, therefore, denser. The appearance is exactly what would result if lateral pressure, due to turgidity, exerted radially in each cell, were to cause condensation

~~along~~ at the ~~line~~ plane of the cell wall, where the pressures from opposite sides would meet.

The fact that the inter-cellular boundaries are often incomplete or indistinct, was noticed by Fernald ('90, p .439), who was inclined to attribute it to poor preservation. I am satisfied, however, that this incompleteness of the side-walls is a normal condition, as my material in which it occurs is in an excellent state of preservation,—showing distinctly the minute alveolation of the cytoplasm.

The nucleus (n.) is situated near the center of the basal half of the cell. It is round or oval, with the long axis in the longitudinal axis of the cell, and measures from four to six micra in diameter. There is a distinct nuclear membrane. The karyoplasm, as well as the chromatin matter, takes the haematoxylin stain much more deeply than does the cytoplasm, becoming dark blue and the chromatin blue-black; while the cytoplasm remains pale blue or takes the contrasting color, if a second stain is used. Numerous chromatin bodies (chr.) and usually a nucleolus (nl.) are present in each nucleus.

Scattered irregularly through the cytoplasm are small round concretions (con.) of various sizes up to one micron in diameter; they are highly refractive, non-stainable, and consist of concentric layers. Except in being smaller, these concretions are precisely similar in appearance to the large pseudo-crystals found deposited in the fat-body. The latter have been described and figured by Sommer ('85, p . 689), who found them insoluble in water and alcohol, but soluble in weak acids with a lively evolution of gas, and concluded that they were calcium carbonate in combination

with some organic substance. Fernald ('90, p . 442) working on Anurida maritima, found similar concretions in two individuals, and concluded that in the rest of his material they had been dissolved by the acids used in fixing and killing.

Willem ('00, p . 101) described the same structures as found in Sminthurus fuscus: "Les concrétions que logent les vacuoles des cellules en question sont sphéroïdales, de taille fort variable, nettement constituées de couches concentriques et conséquemment formées par dépôts successifs autour d'une masse initiale. Insoluble dans l'eau, l'alcool, l'éther, le chloroforme, elles disparaissent dans les acides étendus. Lorsqu'elles sont particulièrement nombreuses, comme chez *Sminthurus fuscus* adulte, leur destruction par une petite quantité de liquide acide s'accompagne de l'apparition de masses cristallines irrégulières qu'on voit s'accroître rapidement sous le microscope; celles-ci présentent par leur aspect, par leur solubilité ou insolubilité respective dans divers réactifs, tous les caractères de l'acide urique. La réaction de la murexide s'obtient d'ailleurs aisément avec des fragments appropriés de Sminthure. D'autre part, si l'on attaque par l'acide chlorhydrique étendu des amas concrétionnaires du même Collembole, on obtient une solution qui, lentement évaporée sur un slide, fournit des cristaux appartenant au système cubique et identiques à ceux du chlorure de sodium."

As the result of my own experiments, I have found that the concretions in the epithelium of the stomach and those in the fat-body behave alike, when treated with water, hydrochloric acid, picric acid, caustic potash, alcohol, xylol, etc. Both kinds are insoluble in alcohol and xylol. After soaking for some time in water,

the concretions assume nearly the same index of refraction as the water, so that they become almost invisible. Prolonged treatment with either acid did not dissolve the peripheral zone, but the central part, which had been more granular, disappeared and must have dissolved, since it could not be recovered in the same form. Treatment with caustic potash for several hours caused the concretions to disappear as such, leaving only pale circular outlines and in some cases, minute granular residues which had formed the nucleus of the concretion.

Notwithstanding Willem's statement that the murexide reaction is easily obtained from the concretions in the fat-body, I failed to get the reaction from either kind of concretions. I did obtain needle-shaped crystals from a hydrochloric acid solution, but am not certain that these came solely from the concretions. Crystals obtained from a picric acid solution of the concretions were needle-shaped and slightly curved, often branching fan-like at one end. These were indistinguishable from crystals of sodic picrate, which I obtained from NaCl. Thus the base of the concretions is sodium; as regards the acid, my partly negative results, I admit, have less weight than the positive results of Willem; indeed, from my own experience, I am inclined to agree with him that the concretions are sodic urate.

The chemical tests used by Willem are the same ones by means of which Plateau ('74, p . 32) obtained sodic urate from the Malpighian tubes of Carabus auratus and Oryctes. Fabre ('56), it may be mentioned, found uric concretions in the mid-intestine of Sphex.

Within the digestive cavity of each of the species examined, it is common to find numbers of spheroidal bodies (Pl. ~~X~~ 2, Figs. 4-12),

varying in diameter from ten to fourteen micra. They have an alveolated cytoplasmic structure not unlike that of the epithelium, and possess the parts of a typical cell, i.e., nucleus (~~n.~~), nucleolus (~~nl.~~) and a distinct nuclear membrane (~~n.m.~~). The nucleus stains more deeply than the rest of the body, and shows distinct chromatin elements (~~chr.~~). These cells are found in all parts of the digestive cavity, on either side of the peritrophic membrane, and also within the epithelial cells (Pl.2,Fig.12,par.), or projecting from the cell as if in the act of passing into the lumen . In the resting cells they may be found on either side of the nucleus, or even wedged between two of the epithelial cells.

These free cells are evidently parasitic, unicellular organisms, probably gregarines. Sommer ('85, p. 715) made the following observation: "Überaus häufig, ja fast als konstanter Parasit findet sich im Chylusdarm eine Gregarine. Dieselbe tritt entweder vereinzelt auf, oder gesellig, zuweilen in solchen Massen, dass statt des Darmepithels fast nur Gregarinen an der Darmwand vorhanden zu sein scheinen, welche mit dem Kopfe in die Zellen eingefressen, dicht an einander gedrängt fast ausschliesslich den Inhalt des Darmes ausmachen.—Zuweilen fand ich auch ausserhalb des Darmes, im ganzen Korper dicht angehauft Pseudonavicellen ähnliche Gebilde.

"Eben so hatte ich Gelegenheit,Entwicklungsstadien von Cestoden, Cysticerken, zu finden, welche den aus dem Darm von Tenebrio molitor von Stein beschriebenen sehr gleichen.

"Endlich ist das Vorkommen junger Nematoden zu erwähnen, welche spiralig zusammengerollt, gelegentlich in mehrfacher Zahl angetroffen wurden." Sommer neither figures nor describes the forms

to which he refers as parasites; I have therefore no way of ascertaining whether or not the organisms which I have observed are the same species that he found. My material, as it happened, contained no Cysticerci or Nematodes.

The intima or lining membrane, is a secretion from the epithelial cells of the stomach. It is striated transversely, the striae being from one to one and one-half micra apart. They are due in all probability to minute pore-canals through which fluids may be either secreted or absorbed. Frenzel, Oudemans, Sommer, Gehuchten and others have described and figured the intima as a layer of fine filaments, to which they give the name "Härchensaum". In Tomocerus, however, the intima presents no such appearance, but is clearly a membrane (Pl. ~~4~~3,Fig.~~6~~16). The thickness of the intima in different individuals varies from that of a barely perceptible line, to a thickness of four micra.

The food-contents (Pl.~~4~~3,Fig.~~6~~16,food) of the stomach are always enclosed in a thin, elastic membrane (per. m.) which holds the substances together in a compact, cylindrical mass. A similar peritrophic membrane, or "funnel", has been described by Schneider ('87, p. 140), as occurring in Thysanura and in many higher forms, such as certain Coleoptera, Diptera, and larvae of Lepidoptera. Schneider observes that "Alle den Trichter besitzenden Insecten (und Larven) fressen feste, selbst unverdauliche Stoffe, während die anderen, flüssige Nahrung zu sich nehmen ."

Various suggestions as to the origin of the peritrophic membrane have been made. Pagenstecher (as quoted by Schneider, p.140) regarded it as a secretion of the salivary glands. No evidence to support such a view is found in the forms I have examined, and the

conception of a tube formed within the stomach, from the secretion of glands in the head, appears to me, to say the least, improbable. Metschnikoff (as quoted by Schneider, p. 140), Oudemans (p. 191), and Schneider (p. 140) state that the peritrophic tube is chitinous, a statement which does not apply in the case of Collembola, for I find, upon treatment with caustic potash for several hours, that the food-enclosing membrane, as well as the intima of the mid-intestine, disappears, while the chitinous intima of the fore- and hind-intestine are unaffected visibly by the reagent. Plateau and Balbiani (quoted by Packard, p. 314) offer a more reasonable explanation, namely, that the membrane is a secretion formed at the surface of the epithelial cells of the chylific stomach, and this I find to be true in Collembola. In almost any specimen, the origin of this membrane around the food may be seen(Pl.~~4~~3,Fig.~~6~~16). The intima either splits or else loosens from the epithelium and envelops the food-mass, at the same time losing its striated appearance, and becoming folded and twisted in accomodating itself to the size of the food-mass when the latter does not fill the lumen. After losing the intima in this way, the epithelium immediately begins to secrete another, which increases in thickness as secretion proceeds.

The food (Pl.1,Fig.1; Pl.~~4~~3,Fig~~6~~16,food) contained in the peritrophic membrane consists mainly of particles of wood, and in many cases, of fungus spores. Three kinds of fungus spores were found repeatedly in different specimens, often in such quantity as nearly to fill the digestive cavity. These were recognizable as Capnodium salicinum, Macrosporium and Triposporium, the last two not being sufficiently intact to admit of specific determination. * † footnote [For the

determination of these fungi, I am indebted to Mr. J. T. Barrett, of the University of Illinois.] In the food mass are found also spherical or elongate bodies (par.), each possessing a nucleus, nucleolus and numerous vacuoles, which bodies appear to be identical with those before described as embedded in the epithelium, and as being probably gregarine parasites.

In the stomach cavity and outside the peritrophic membrane there is usually a faintly staining, evenly granular substance (pl.) similar to the blood-plasma found in the body cavity, but containing no corpuscles nor nuclei. This substance is probably a digestive fluid secreted by the epithelium of the stomach.

Stage II. (Pl.4, Fig.17)

An insect killed about forty-eight hours before ecdysis, exhibits the following modifications of the epithelium. The alveolation has become somewhat coarser, which brings about an increase in the length of each cell and consequently in the thickness of the whole epithelial wall. The highly refractive granules, or concretions (con.) in the cytoplasm become more numerous, especially in the region between the nucleus and the intima.

A series of important changes in the epithelium now occurs. These changes are continuous and rapid, and the entire series is completed by the time the external moult takes place. From this stage until the next resting stage, the side walls (lat.) of the cells are indistinct, except occasionally for a short distance near the base, the cells being distinguished from one another only by their nuclei and by differences in density.

Stage III. (Pl. 4,Fig.19).

The thickness of the epithelial wall is now from thirty-six to forty micra,—an enormous increase as compared with its thickness in the resting stage,—and the inner half of the cell shows numerous large vaculoes ~~(vac.)~~ from three to ten micra in diameter, many of which contain conspicuous concretions (con.). A distinct row of vacuoles (vac.), four to six micra in diameter, is found immediately beneath the intima (in.). The gregarine parasites (par.) are now found in the ental half (ent.) of the wall, and in most cases, each is surrounded by a clear vacuolar space.

The most striking difference, however, between this stage and the prece~~e~~ding, is seen in the position of the nuclei ~~(n.)~~ mig. n, some of which have moved from their original position in the basal half (ect.) of each cell, to a new position in the more coarsely vacuolated area (ent.) nearer the lumen, forming a second layer of nuclei, inside the original, or primary, layer. The number of migrant nuclei (mig. n.), is somewhat less than the number of remaining nuclei, being about forty-three per cent. of the whole number. It is noticeable in some cases that each of the migrant nuclei is entirely surrounded by large vacuoles, as it moves from the basal to the ental half of the cell. But what moves the nuclei? I believe that this nuclear migration is to be explained in terms of cell-vacuolation, the changes in which have been followed closely in this study, as appears in the figures given. Immediately after the nuclear division which took place at the preceding ecdysis (as described beyond), the two daughter nuclei remain near each other, though separated by a cell-wall. The plane of division is such as to leave one of the daughter nuclei somewhat nearer the

intima than is the other (Pl.~~7~~ 4,Fig.~~8~~ 18). As ecdysis approaches, the inner half of the cytoplasmic layer becomes less dense than the outer half, owing to the distention of its alveoli and vacuoles, and the daughter nuclei which are nearer this region of lesser density, move into it. This movement is accounted for by the turgidity of the cytoplasm. When a cell of the stomach epithelium is isolated, in its own fluid, without the addition of water, it swells to a spheroidal form, owing to its turgidity (Frenzel,p.259, Tab. 7, Fig. 9; Cudeman s, p. 190, Tab.III,Fig. 36), and this same quality seems to explain the propulsion of a nucleus from a region of greater density to one of lesser density. It explains as well, the removal of gregarines from the cells of the stomach, and also the removal of excretory concretions (as described beyond) from the same cells.

Stage IV. (Pl. ~~6~~ 5, Fig. ~~10~~ 20).

Following the condition described above, a separation appears between the denser, basal part (ect.) of the epithelial wall and the more coarsely vacuolated portion (ent.) next the lumen. The separation is effected by a dividing membrane (in.) which forms midway between the intima and the basement membrane. This dividing wall is at first extremely thin, but it gradually increases in thickness, and acquires all the appearance of the ordinary intima. As it grows in thickness, it pushes before it the vacuolated ental division of the wall.

The number of nuclei (n.) remaining in the primary position, i.e., near the basement membrane, is now restored to the normal number by mitotic division. Since the number before this division

is rather more than half the original number, and since the number of cells in the resting epithelium of the adult insect does not increase from moult to moult, it follows that not all the nuclei left behind divide, but only enough to replace those which were lost; to express it in this way. In the growing insect, however, there occurs an actual increase in the number of epithelial cells. The nuclear division is indirect or mitotic, as was said. Immediately before division, the nucleus (Pl. ~~6~~ 4, Fig. ~~9~~ 19) becomes slightly larger, (seven to eight micra in diameter) than in the resting stage (five to six micra), the karyoplasm becomes more coarsely reticulated, or alveolated, and the chromatin elements stain in sharper contrast to the karyoplasm. The various phases of mitosis (Pl. ~~7~~ 5, and 6, Figs. ~~11-18~~ 21-28), are distinct and easily recognized, especially in material stained with haematoxylin and Orange G; yet the chromatin bodies are small, and crowd together in such a way as to make it almost impossible to determine the exact number of chromosomes. In some instances, however, (Pl. ~~7~~ 6, Fig. ~~15~~ 26, 27) the number of chromosomes appeared to be sixteen, arranged in eight pairs. The plane of division is transverse to the axis of the stomach. Insects killed just after the division of the epithelial wall into two layers (Pl. ~~6~~ 5, Fig. ~~10~~ 20), invariably show mitosis in full progress. In some instances, a count of all the nuclei which appeared in a tangential section of the outer part of the wall showed fully thirty per cent. of the nuclei to be involved in one or another stage of mitosis. Immediately after division the daughter nuclei (Fig. ~~10~~ 20, dau. n.), are smaller (three to five micra in diameter), and stain more intensely than do the nuclei in the resting stage.

Stage V. (Pl. ~~8~~ 6, Fig. ~~20~~ 30)

Following the separation of the epithelium into two layers, the outer layer (ect.) gradually decreases in thickness and returns to the resting condition. The inner part (ent.) consisting of loosely organized cytoplasm, and containing nuclei (deg. n.), concretions (con.) and parasites (par.), becomes enclosed in a membrane precisely similar in structure and origin to that which encloses food in the stomach; it is, then, another peritrophic membrane. The whole mass, which since Stage IV. has occupied the entire digestive cavity (Pl. ~~8~~ 6, Fig. ~~18~~ 24), now gradually shrinks (Pl. ~~8~~ 7, Fig. ~~22~~ 32) and the cytoplasm undergoes marked changes in appearance (Pl. ~~8~~ 7, Fig. ~~21~~ 31). The alveoli (alv.) and vacuoles ~~(vac.)~~ coalesce and finally break down, leaving only fragments of cytoplasm which have lost all alveolation and become merely granular, and which for the most part, eventually disappear. The nuclei offer, apparently, more resistance to the process of degeneration than does the cytoplasm, retaining their identity after the alveolation of the cytoplasm is lost. They become, however, slightly swollen (Pl. ~~3~~ 2, Figs. ~~4~~ 13-15, ~~deg. n.~~), and lose the nuclear membrane. The chromatin bodies (chr. Fig. ~~4~~) of each nucleus remain for a time in their relative positions, but finally become separated from one another and scattered about, or else aggregated in irregular masses. The concretions (Pl. ~~8~~ 6, Fig. ~~20~~ 30, con.) alone, of all the elements which were formerly a part of the epithelium, are unaffected by the process of degeneration. Parasites, if present, also remain unaltered and are cast out of the alimentary canal, but these, of course, have never been in a true sense, a part of the epithelium. The residue which is ejected from the alimentary canal at once after ecdysis, consists almost

entirely of the concretions already referred to, with the fragments of chromatin matter, all massed solidly together.

V. REGENERATION.

The process of degeneration which I have described is quite distinct from the regeneration of the epithelium of the mid-intestine, which latter process has been fully described by Frenzel for Coleoptera, Diptera, Lepidoptera and Hymenoptera. He says ('85, p. 293): "Begegnet man auch in den Schnittpräparaten, besonders in denen der Hymenopteren und Schmetterlingslarven, einer Anzahl kleinerer basal im Epithel sitzender oder allmählich in die Höhe rückender Zellen, die man sofort als Jugendformen ansehen wird. Woher diese Zellen kommen und wie sie entstehen, soll hier nun weiter untersucht werden.----Es wird sich zeigen, dass man folgenden Satz aufstellen kann: Die eigentlichen Epithelzellen im Mitteldarm der Insekten, gleichgültig ob sie dem Darmschlauch selbst oder auch dessen Ausstülpungen angehören, gleichgültig ferner ob sie dem Typus der langgestreckten Cylinderzellen oder den der rundlichen Schleimzellen zuzuzählen sind, pflanzen sich auf dem Wege der direkten (amitotischen) Kerntheilung (Holoschisis) fort, während die spezifischen Drüsenzellen der Krypten sich auf dem Wege der indirekten (mitotischen) Kerntheilung (Karyolyse) vermehren." Faussek ('87, p. 697) finds the regenerative process in Eremobia and Aeschna larvae to be similar to that described by Frenzel, in so far as he (Faussek) was able to observe. Turning to Thysanura, to which Collembola are most nearly allied, we find Oudemann's reference to the process in Machilis, as follows ('87, p. 192): "Alle meine Beobachtungen sprechen für eine Regeneration des Epithels in

den tiefen Stellen: (1) sind die Zellen dort am kleinsten und gehen *gleichmässig* in die grösseren über; (2) finden (indirekte) Kerntheilungen in den am tiefsten liegenden Zellen Statt; (3) ist die Quantität der ausgeschiedenen Secrettropfen grösser, je weiter die Zellen von den tiefen Stellen entfernt sind und (4) lassen die Randzellen der Polygonen leichter los und zerreissen auch leichter als die Zellen in der Tiefe."

In Collembola, as compared with other insects, the conditions are much simpler; for the regeneration of the epithelium does not proceed from local aggregations of cells, but takes place throughout the epithelial layer, by means of cell-division, as I have observed. Furthermore, the nuclear division is mitotic in Collembola, instead of amitotic as it is said to be in other insects.

VI. SECRETION.

The epithelium of the middle region of the stomach is marked by the frequent occurence of cells (Pl. ~~9~~7, Fig. ~~23~~33, sec.c.) the contents of which are conspicuously clear, on account of the excessive amount of vacuolar fluid and the reduced amount of inter-vacuolar substance. Scattering cells of this type are found on all sides of the lumen and as far down the alimentary tract as the posterior part of the third abdominal segment, but they are most numerous on the ventral side, and in the region of the first abdominal segment. In specimens which had not eaten for some time previous to being killed, these cells were so distended with fluid as to project for a distance of two or three micra into the stomach cavity, and the vacuolar contents were dense enough to crowd the nucleus to one side or to the base of the cell. These clear cells, which I have

[illegible]

[illegible]

[illegible]

[illegible]

observed also in the stomachs of other insects (as the larva of Bombyx mori) are specialized for the purpose of secreting a copious fluid, which accumulates in the cell when no food is taken into the stomach, but otherwise passes through the intima to mix with the food.

In insects which had fasted for five or six days, large vacuoles occurred around the nucleus (Pl. ~~10~~ 7, Fig. ~~24~~ 34, vac.) and often a conspicuously large vacuole (Pl. ~~10~~ 6, Fig. ~~25~~ 35 vac) (four to six micra in diameter) occurred in each cell, near the intima. These vacuoles also, I infer, contain accumulated digestive secretions. In a few instances, after the insects had fasted, the epithelial wall was found to be exceptionally thin (Pl. ~~10~~ 8, Figs. ~~26~~ 36, ~~27~~ 37), on account of a reduction of cytoplasmic substance, probably by absorption into the blood.

The epithelium of the posterior part of the mid-intestine, (Pl. ~~11~~ 9, Fig. ~~28~~ 38), is marked by numerous proliferations which appear on the ental surface of the cells and project into the lumen. Since these proliferations are found in various stages in the same individual, it is not difficult to trace their origin and development. The cells of this region are long and narrow and are crowded together more closely than those of the middle and anterior regions of the stomach. A cell of this kind, turgid with digestive fluid, becomes convex on its free surface (Fig. ~~28~~ 38, sec. ~~p.1, 2~~ p^1, p^2). This convexity increases, carrying the intima before it, until a part of the cell projects into the lumen (sec.p.^{3}3.). Next, the connection between the projection and the cell constricts sec. 4 (4) until there results the separation of a spherical body sec (~~5~~), six to eight micra in diameter, exclusive of intima, made up of ordinary cytoplasm

frequently containing concretions but invariably without a nucleus. After becoming free in the digestive cavity, the spherical body loses its intima (in.), becomes less definite in outline and stains less deeply. The vacuoles and alveoli break down, their contents escaping to mix with the food. The residue of reticular substance remains for a short time faintly granular, but soon disappears. It appears that this process also is one whereby digestive fluids which have been elaborated by the cell, are given off to act on the food.

Gehuchten has described the process of secretion in the larva of ptychoptera. According to his observations(as quoted by Packard, pp. 326-329), when an epithelial cell is about to empty its secretion into the alimentary canal, the cell becomes distended and a part of the cytoplasmic body projects into the digestive cavity. A glandular cell of the chylific stomach, when at rest, is furnished with a striated "platform" on the digestive surface, the platform being provided with filaments. But when active, the cells present a very different appearance, since in many of them the platform has disappeared, and is replaced by a simple membrane. During the process of secretion, a finely granular mass, continuous with the cytoplasm, swells, raising the limiting membrane over the entire breadth of the cell, and causing it to project into the lumen. A pear-shaped vesicle is thus formed, which eventually is constricted off from the cell and set free in the lumen of the stomach. These vesicles, both before and after separation from the cell, are clear and transparent in the living insect, but more granular in the tissue fixed for sectioning. Gehuchten then describes a modification of the process which occurs when

secretion is most active. The limiting membrane may then be raised in several places at once on a single cell, and several vesicles may form instead of one. After becoming detached from the cell, these vesicles rupture and their contents mix with the food.

Needham ('98, pp. 103-113) has described the accumulation and discharge of the digestive secretion in dragon-fly nymphs, in which the digestive epithelium is of the same type as in Orthoptera. Interspersed between the cylindric cells are groups, or "nidi" of small, roundish cells, close to the basement membrane, and having no communication with the digestive cavity. The secreting process consists in the accumulation of granular material in the cells midway between the nidi. These cells then rise in elevations with turgid summits from which the striated border has disappeared, and a globule is discharged as if crowded out by compression from adjacent cells. Each globule discharged represents the larger part of a cell, including,generally,the nucleus. Needham agrees with most other recent investigators, in considering the nidi to be centers of regeneration in which new cells arise (p. 113)"by division, grow, crowd their way to the surface, acquire a striated border, become functional, secrete, discharge, digest and die, giving place to others which in turn run the same course."

Certain differences will be seen at once between the processes of secretion as described by Gehuchten and Needham, and the process in Collembola, since in the latter the proliferation does not burst through the intima, but is still covered by the striated membrane when constricted off. Moreover, in Collembola the discharged portion does not represent the greater part of the cell, nor does it ever include the nucleus, as in the dragonfly nymph.

VII. CONCLUSION.

What is the meaning of the periodical degeneration of half the epitheilial wall of the mid-intestine? Sommer has suggested one use of the cast-off epithelium. He says, (p. 715): "Ob die abgestossenen Zellen dem Thiere während der Häutung, wo dasselbe keine Stoffe von aussen aufnimmt, zur Nahrung dienen, lässt sich mit Bestimmtheit nicht behaupten, ist aber nicht unwahrscheinlich." This view is supported by the decrease in quantity of the cast-off material before it is finally ejected; for the evident explanation of this decrease is that the nutritive elements are digested and resorbed. This is merely incidental to a larger process, however, which must be accounted for. While the insect is growing, the growth of the stomach is effected by a constant increase in the number of its cells, through mitotic division. A simple explanation, then, would be that this phenomenon is merely an incidental result of the continuance of the ordinary process of cell-division after the stomach has reached its maximum size; when, therefore, the surplus cells with their nuclei must be disposed of. It is evident, however, that the phenomenon is something more than an abnormal prolongation of ordinary cell-multiplication, from the fact that it is not confined to full grown individuals, but occurs throughout the life of the insect.

A second possibility that suggests itself is that the phenomenon is a secretory process. It is already known that among insects of several diverse orders (see p.22) some of the cells of the stomach cast off each a portion of its cytoplasm in the form of a rounded vesicle, the contents of which mix with the food in the lumen of the stomach. Have we here essentially the same thing,

except that it involves the entire epithelial wall, instead of being local, and involves nuclei as well as cytoplasm? The answer is,--no; for at the time of the casting-off of the epithelium until its remains are discharged from the rectum, there is usually no food whatever in the alimentary canal, and at most, only the slightest traces of food.

The most reasonable interpretation at which ~~I~~ we have been able to arrive is the following,-- an induction from the facts observed. Soon after a moult, when feeding is resumed, or even before food is eaten, peculiar concretions (see p.) appear in the cells of the stomach. These concretions increase in number continually. They are scattered throughout the cell until it is nearly time for the moult; then they accumulate in the ental half of the cell, which is soon cut off from the rest of the cell, and cast, with its contents, into the lumen of the stomach, where the concretions remain intact until they are ejected from the alimentary canal. These concretions form in the cell as products of cell-metabolism; they increase with the age of the cell; they are insoluble in the blood of the insect, and are highly resistant to ordinary chemical reagents, (see pp. 9-10); they eventually load the cell, become segregated at the inner end of the cell and are then cast off. Therefore I regard them as excretory products. Being insoluble, they are removed bodily, and this removal is accomplished by cell-division. Though the removal involves an initial sacrifice of half the cytoplasm, and half the number of daughter nuclei, the fluid contents of both these are resorbed by the remaining epithelium, before the concretions, chromatin matter and fragments of disintegrated reticular substance are expelled from the body.

Concretions of precisely the same kind, though larger, are deposited in the fat-body of Collembola, where they increase in number with the age of the insect and where they remain until the insect dies.

If these concretions are indeed uric acid or one of its derivatives, as Willem maintains, that is additional and most important evidence in favor of my excretory theory.

This conclusion-that the stomach in Collembola has an excretory function,— is of course unique for insects. But Collembola themselves are unique among insects (excepting possibly Japyx) in having no Malpighian tubes for the extraction of waste products from the blood,—and excretion is one of the vital necessities of any cell.

VIII. SUMMARY.

In Collembola, a degeneration of the inner half of the epithelial wall of the mid-intestine occurs in connection with each ecdysis. The cells of the mid-intestine become confluent and important changes of alveolation ensue; nearly half the nuclei migrate toward the intima, while the rest of the nuclei remain near the basement membrane; a wall now forms between the two sets of nuclei, dividing the epithelium into two concentric layers. The inner of these two layers degenerates; the cytoplasmic reticulum disintegrates; the nuclear membranes disappear and the chromatin granules become scattered, but remain intact; much of the fluid substance is resorbed into the remaining layer of cells. The disorganized epithelium, surrounded by a peritrophic membrane, is expelled through the rectum shortly after the external moult.

The process is an excretory one. By this means, the rapidly accumulating concretions of sodic urate are removed from the cells of the mid-intestine, as are also, but incidentally, unicellular parasites (Gregarinidae).

The nuclei lost by degeneration are replaced by the mitotic division of the remaining nuclei,--this occurring before the inner portion of the epithelium is cast off.

The peritrophic membrane, which always envelops a food-mass, is formed by the splitting of the intima, and is therefore a secretion from the epithelium of the mid-intestine. The wall that divides the originally single layer of cells into two layers, splits into two membranes, one of which surrounds the degenerating epithelium as a peritrophic membrane, while the other forms the new intima of the mid-intestine.

The formation of new cells takes place throughout the epithelium, by mitosis; this regeneration does not occur from local centers, or "crypts", as it does in other insects; furthermore, no amitotic divisions are found at any time.

Secretion is performed (1) by the general epithelium of the mid-intestine; (2) by special clear cells in the middle region of the stomach; (3) by specialized cells in the posterior region; these last give off proliferations into the lumen, which become constricted off, as free, rounded, cytoplasmic vesicles, which break down in the alimentary canal and mingle their contents with the food.

This novel rôle of the mid-intestine as an organ of excretion is correlated with the absence of Malpighian tubes in Collembola.

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X. EXPLANATION OF PLATES.

All figures are of Tomocerus niger Bourl. Outlines of drawings were made with an Abbé camera lucida, and unless otherwise specified, were magnified 750 times and drawn three times this size. Detail is represented as seen with a 1/10 homogeneous oil-immersion objective.

~~XI. LIST~~ OF ABBREVIATIONS.

alv.	Alveoli. ✓ l.c.
b. m.	Basement membrane. ✓ l.c.
car.	Cardiac valve. ✓ l.c.
chr.	Chromatin body. ✓
c. m.	Circular muscle. ✓
con.	Concretion. ✓
dau. n.	Daughter nucleus. ✓
deg. cyt.	Degenerating cytoplasm. ✓
deg. n.	Degenerating nucleus. ✓
ect.	Ectal division of cytoplasm. ✓
ent.	Ental division of cytoplasm. ✓
food.	Food mass. ✓
f. int.	Fore-intestine. ✓
h. int.	Hind-intestine. ✓
in.	Intima. ✓
l.	lumen.
lat.	Lateral wall. ✓
l. m.	Longitudinal muscle. ✓
mig. n.	Migrant nucleus. ✓
m. int.	Mid-intestine. ✓
n.	Nucleus. ✓

n. m. ---------------Nuclear membrane. ✓

nl. -----------------Nucleolus. ✓

par. -------- -- ---Parasite. ✓

per. m. ------------Peritrophic membrane. ✓

pl. -----------------Plasma. ✓

pyl. ----------------Pyloric valve. ✓

sec. c. -------------Secretory cell. ✓

sec. p. -------------Secretory proliferation. ✓

vac. ----------------Vacuoles. ✓

Plate 1.

Fig. 1. Sagittal section of Tomocerus niger. × 112.

Plate 2.

Fig. 2. Epithelium of mid-intestine at Stage 1 (resting stage); radial section of mid-intestine.

Fig. 3. Outlines of cells of mid-intestine, in cross section. × 630.

Figs. 4-11. Gregarines in various conditions.

Fig. 12. Gregarina (par) in epithelial wall of mid-intestine of an insect which had fasted for six days; radial section.

Figs. 13-15. Disintegrating nuclei found in the alimentary canal.

Plate 3

Fig. 16 — Portion of a radial section of the mid-intestine, showing origin of peritrophic membrane from intima. × 630.

Plate 4

Fig. 17. Epithelium of mid-intestine at Stage 2; radial section.

Fig. 18. Daughter-nuclei in alternating positions; radial section.

Fig. 19. Epithelium of mid-intestine at Stage 3; radial section.

Plate 5

Fig. 20. Epithelium of mid-intestine at Stage 4; radial section.

Figs. 21-25. Phases of mitosis in nuclei of epithelium of mid-intestine. Fig. 21, metaphase; Figs. 22-25, anaphase.

Plate 6

Figs. 26-28. Phases of mitosis in nuclei of epithelium of mid-intestine. Figs. 26, 27, anaphase; Fig. 28, telophase.

Fig. 29. Sketch to show a portion of the digestive cavity occupied by cast-off epithelium. Stage 4; radial section. × 547.

Fig. 30. Epithelium at Stage 5; radial section.

Plate I.

Fig. 1. Sagittal section through entire animal.

X 140.

Plate I.

Plate 7

Fig. 31. Degenerating cytoplasm. ×547.

" 32. Degenerating cytoplasm enclosed in peritrophic membrane. ×123.

Fig. 33. Secretory cells in middle region of mid-intestine; radial section.

Fig. 34. Cell from mid-intestine, after fasting; radial section.

Plate 8

Figs. 35–37. Cells from mid-intestine, after fasting; radial section.

Plate 9

Fig. 38, 39. Epithelium of posterior region of mid-intestine, to show the development of secretory proliferations; radial sections.

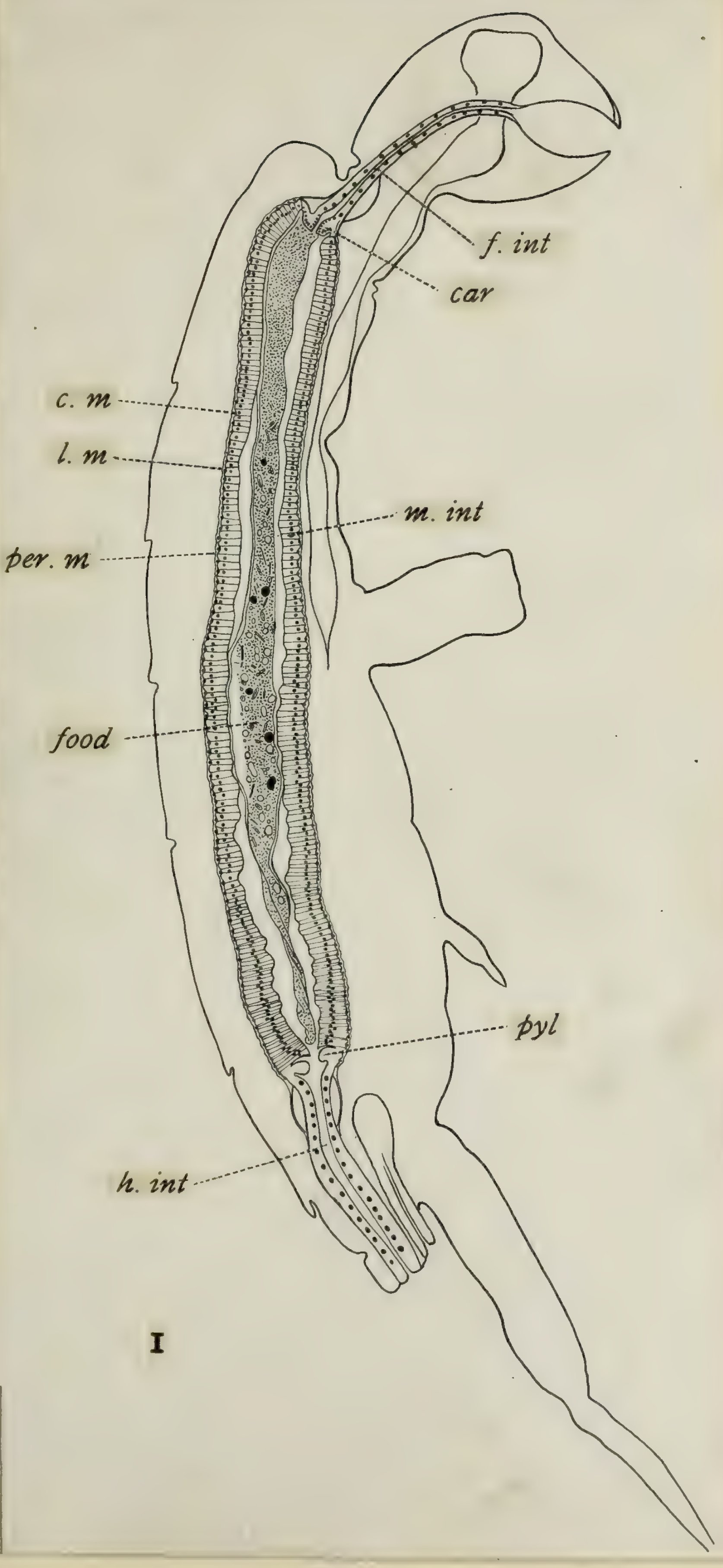

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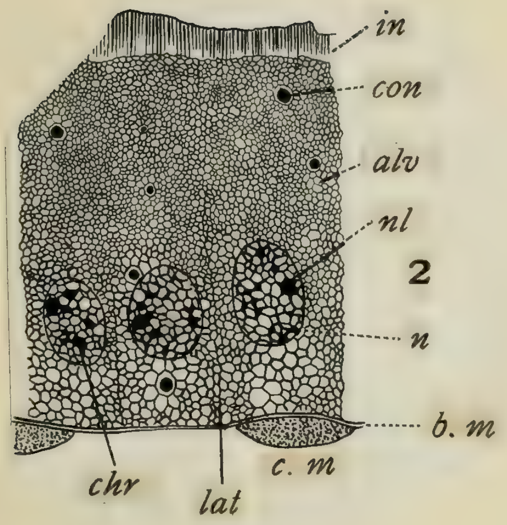
Plate II.

Fig. 2. Epithelium of mid-intestine at Stage I (resting stage

Fig. 3. Outline of cells in cross section. × 750.

Plate II.



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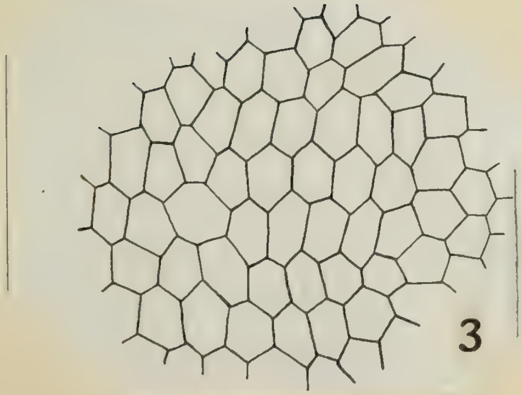

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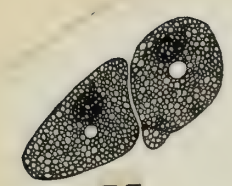
Plate III.

Fig. 4. Gregarines and disintegrating nuclei of digestive cavity.

Fig. ~~5~~. 12 Gregarine in epithelium. (Insect had been deprived of food for six days previous to killing.)

Plate III.



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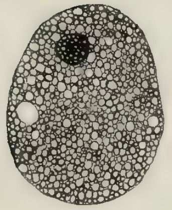

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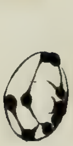

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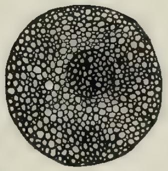

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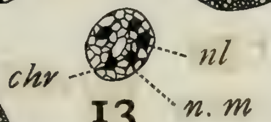

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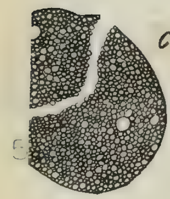
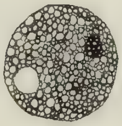
Fig. 8





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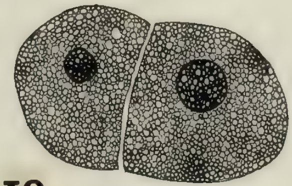

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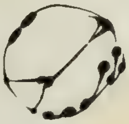

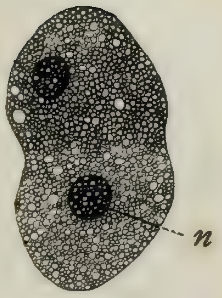

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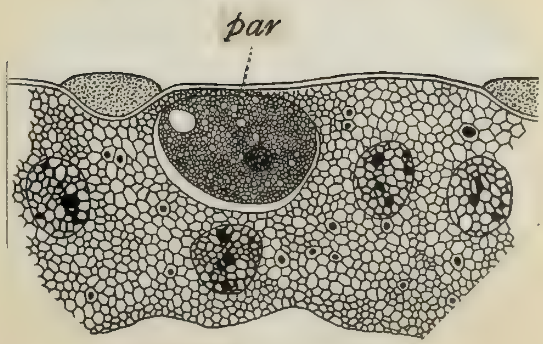

Fig. [illegible]

Plate IV.

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Fig. 6. Section showing origin of peritrophic membrane from intima. X 750.

Plate IV.

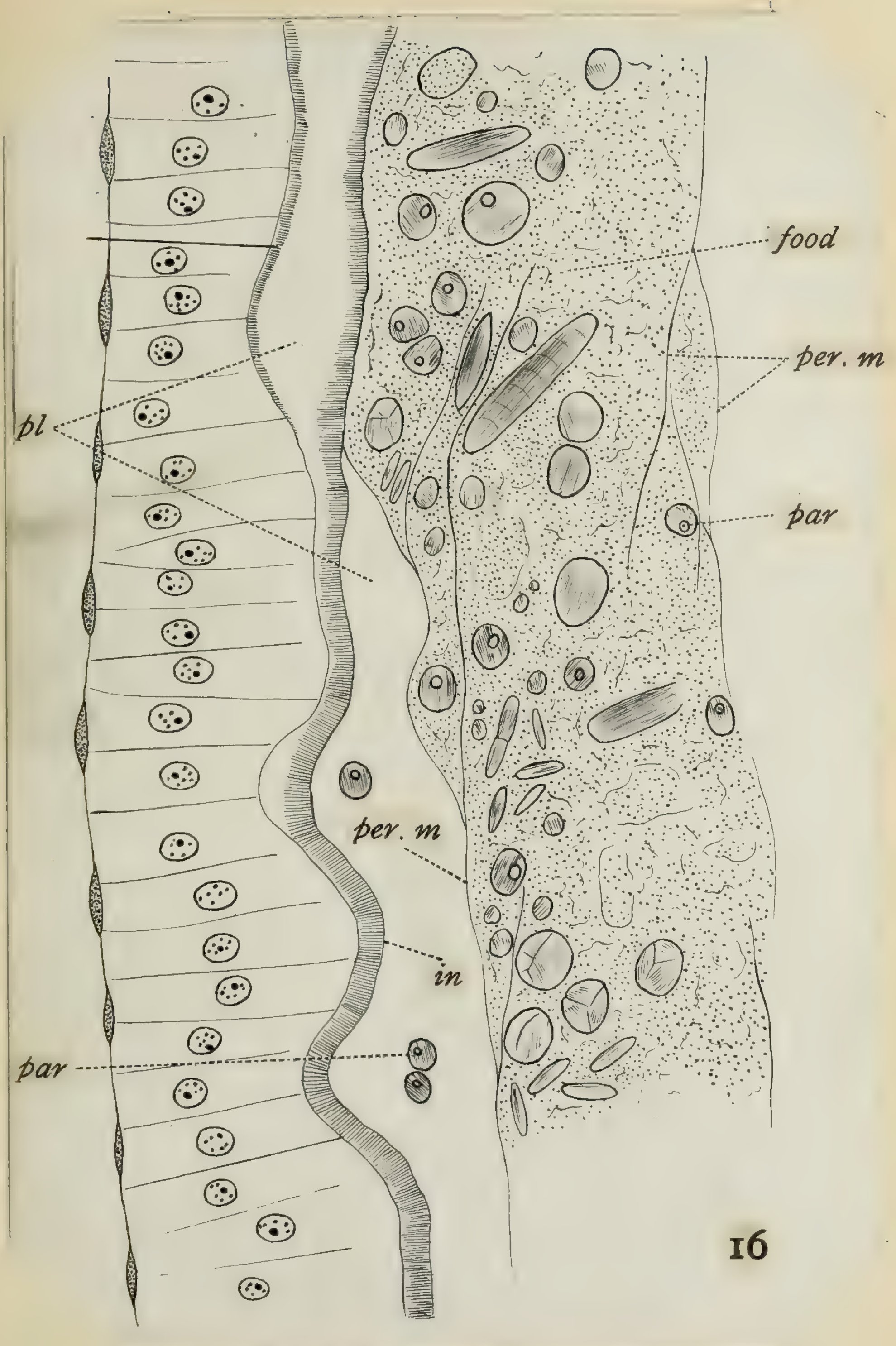


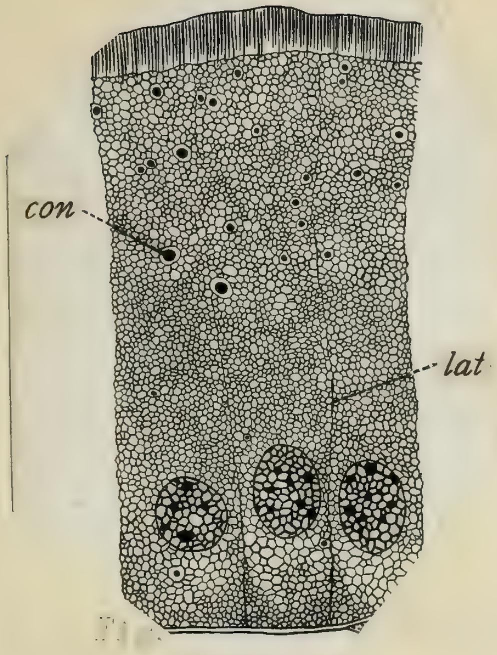
Fig. 5.

Plate V.

Fig. 7. Epithelium of mid-intestine at Stage II.

Fig. 8. Daughter nuclei in alternating positions, those nearer the intima being at a lower focus than those nearer the basement membrane.

Plate V.



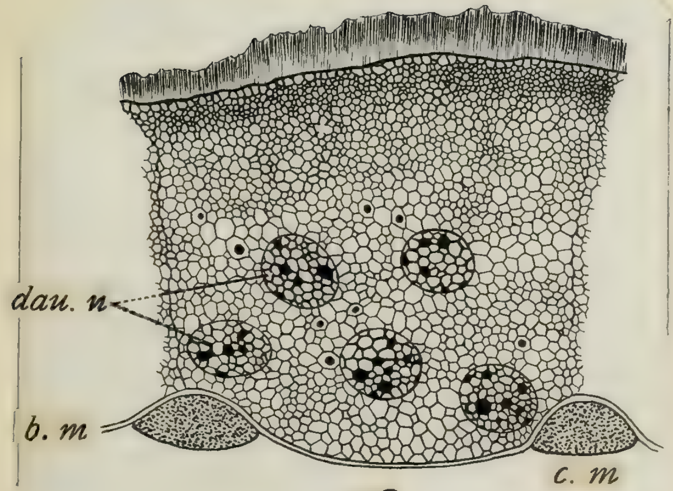


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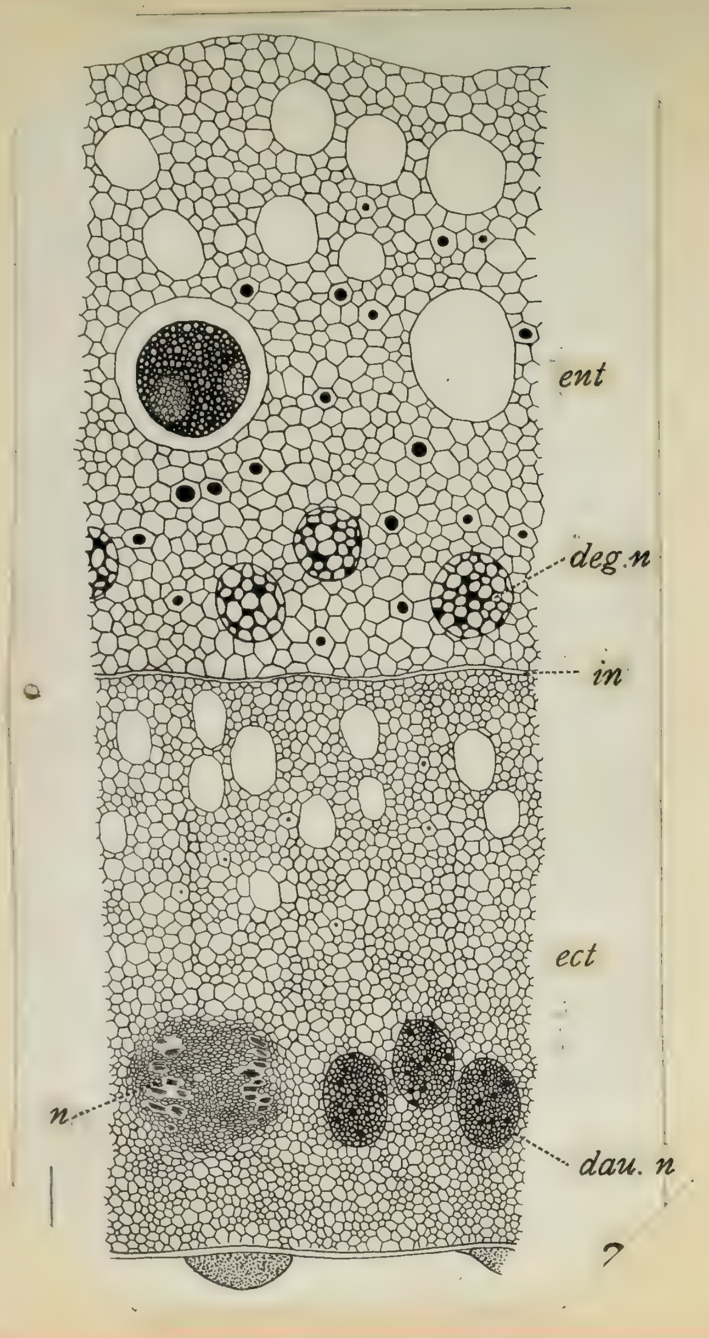
Plate VI.

Fig. ~~9~~ 11. Epithelium of mid-intestine at Stage III.

Fig. ~~10~~ 12 Epithelium of mid-intestine at Stage IV.

Plate VI.



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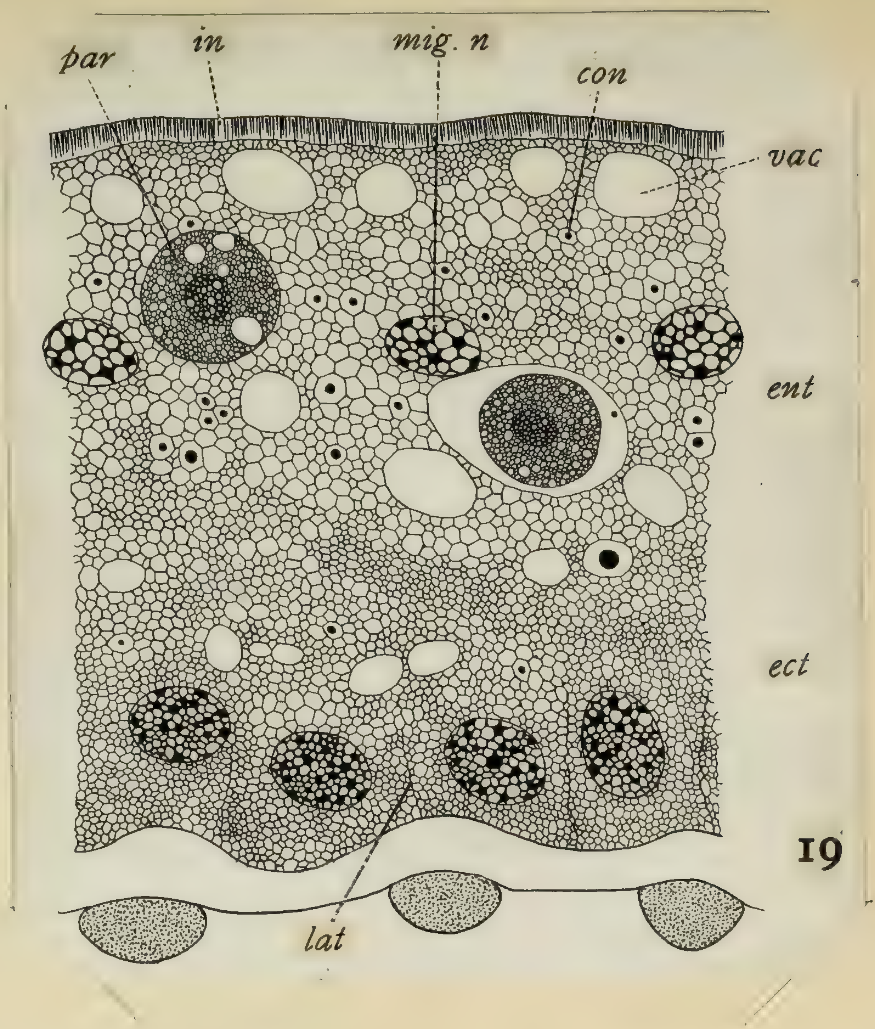

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Plate VII.

Phases of mitosis in nuclei of epithelium of mid-intestine.

Fig. 11. Metaphase.

Fig. 12. Anaphase.

Fig. 13. "

Fig. 14. "

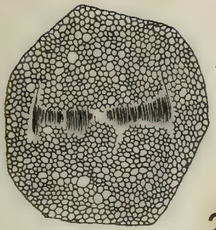
Fig. 15. "

Fig. 16. "

Fig. 17. "

Fig. 18. Telophase.

Plate VII.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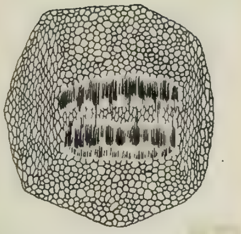
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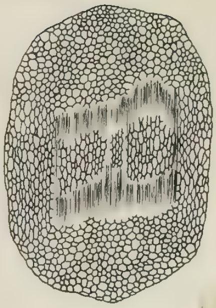
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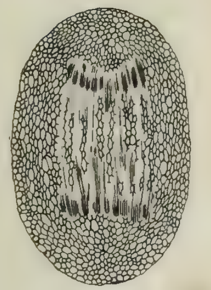
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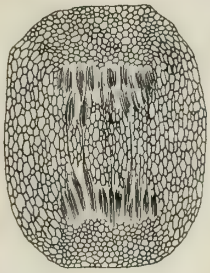
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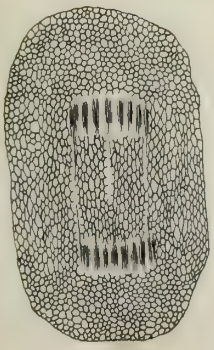
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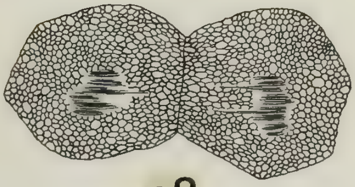
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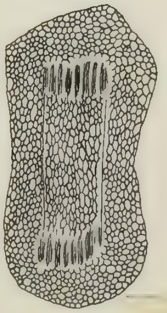
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Plate VIII.

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Fig. ~~1~~9. Sketch to show digestive cavity entirely occupied by cast-off epithelium, Stage IV. X 650.

Fig. ~~20~~. Epithelium at Stage V.

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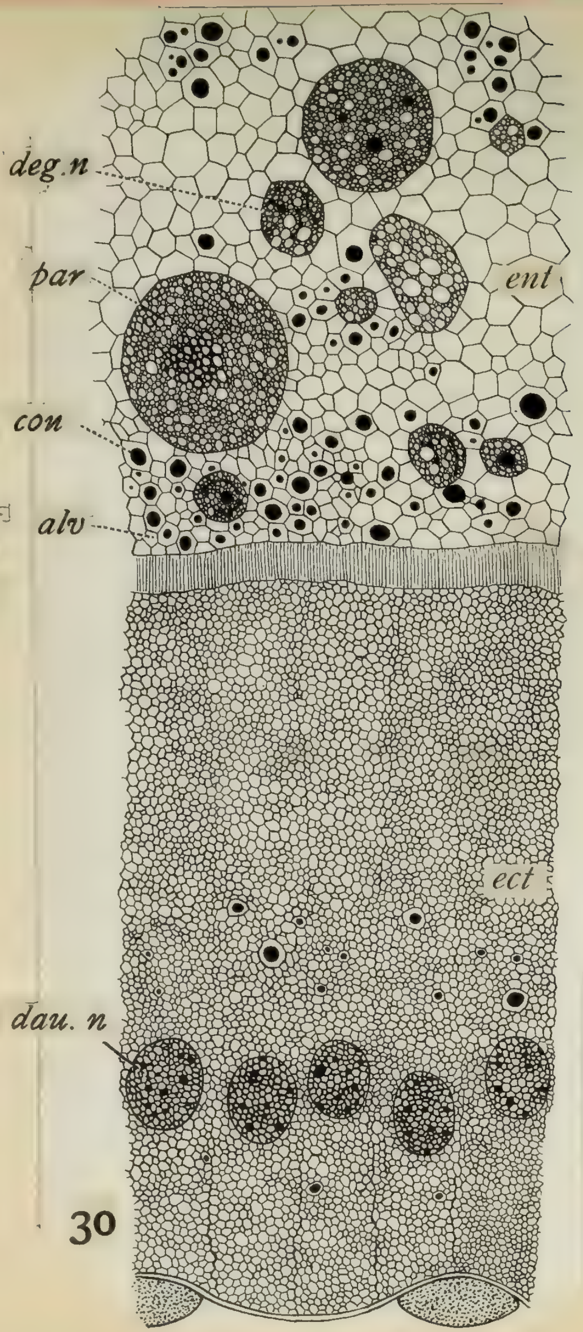
Plate VIII.



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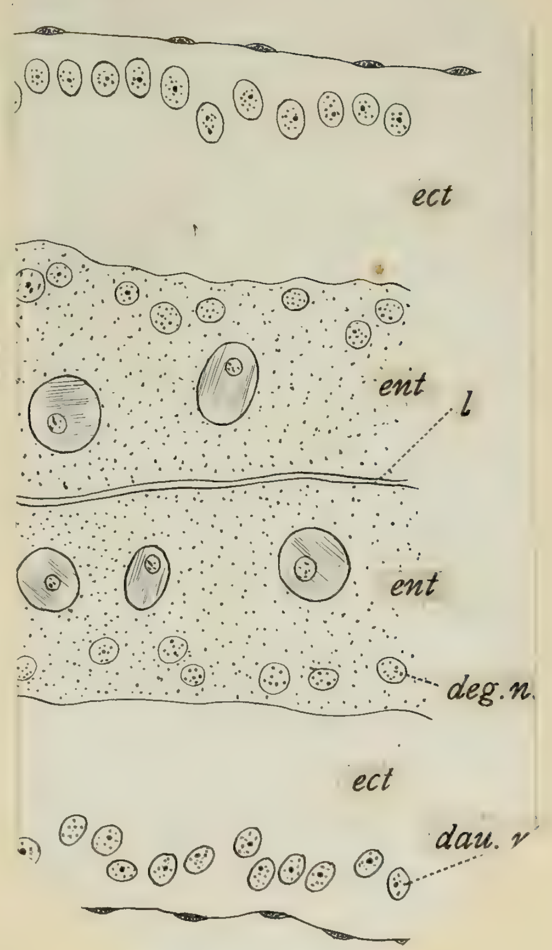


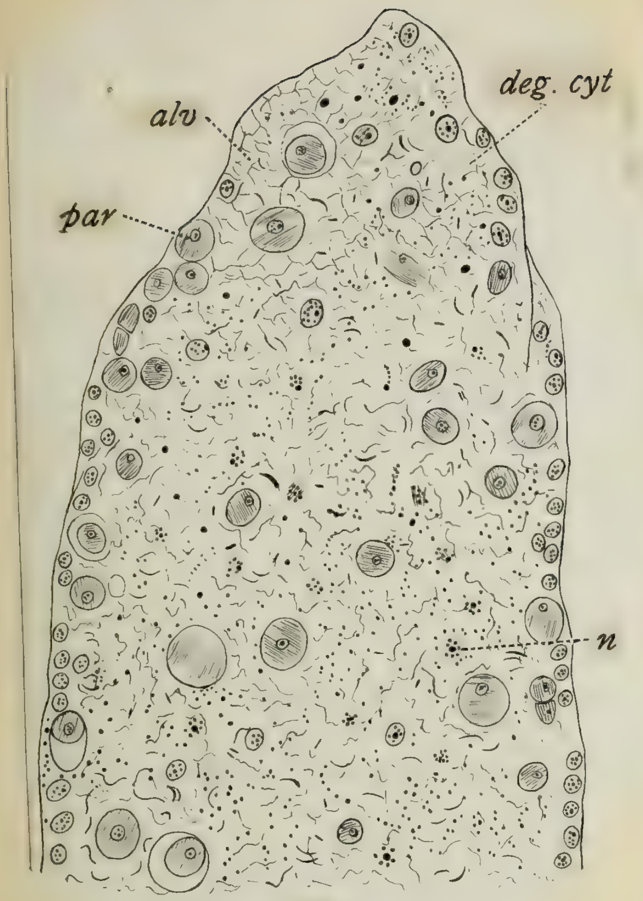
Plate IX.

Fig. 21. Degenerating cytoplasm. X 650.

Fig. 22. Sketch showing degenerating cytoplasm enclosed in peritrophic membrane. X 146.

Fig. 23. Secretory cells in middle part of mid-intestinal wall.

Plate IX.



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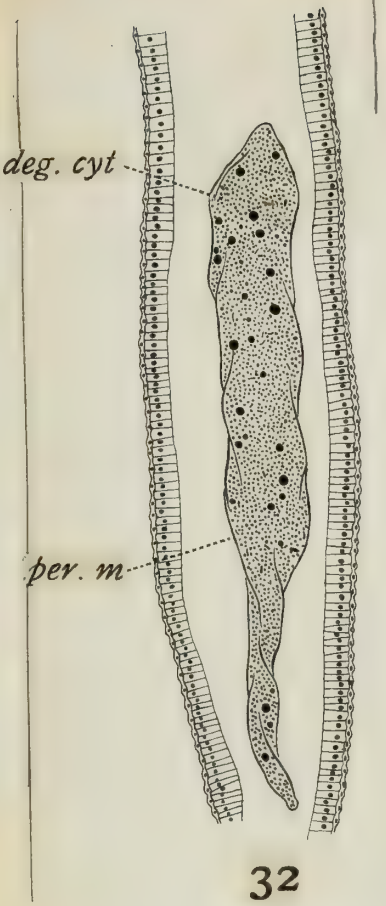

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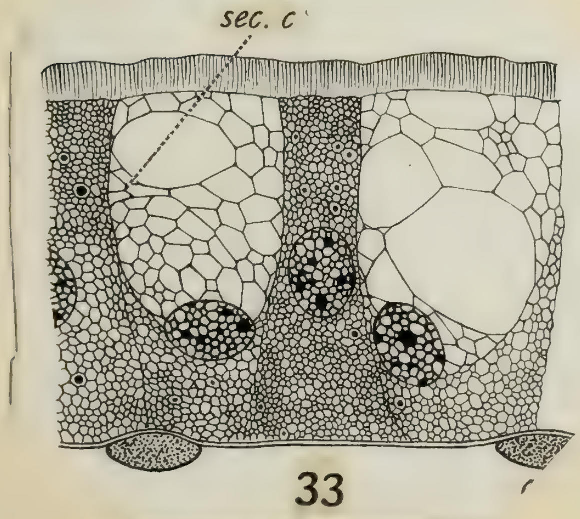

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Fig. 33.

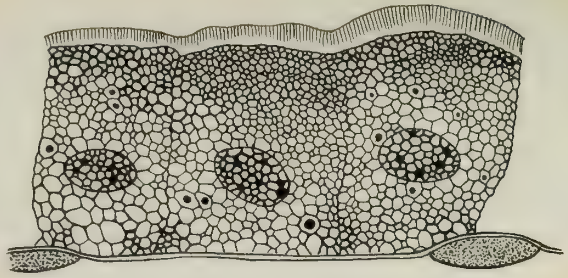
Plate X.

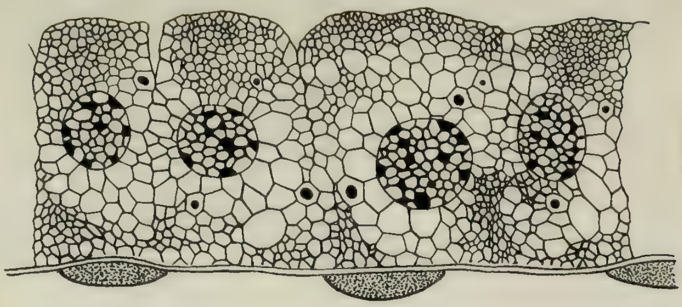
Fig. 24. Cells from mid-intestine after fasting.

Fig. 25. " " " " " "

Fig. 26. " " " " " "

Fig. 27. " " " " " "





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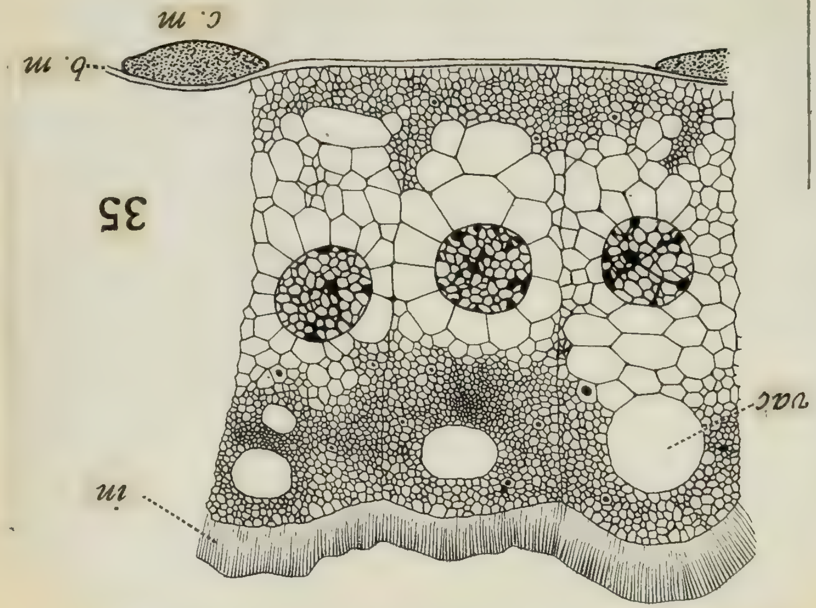

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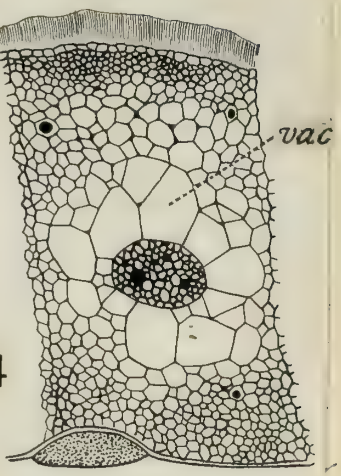

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Plate X.

Plate XI.

Fig. 28. Epithelium of posterior region of mid-intestine.

Plate XI.

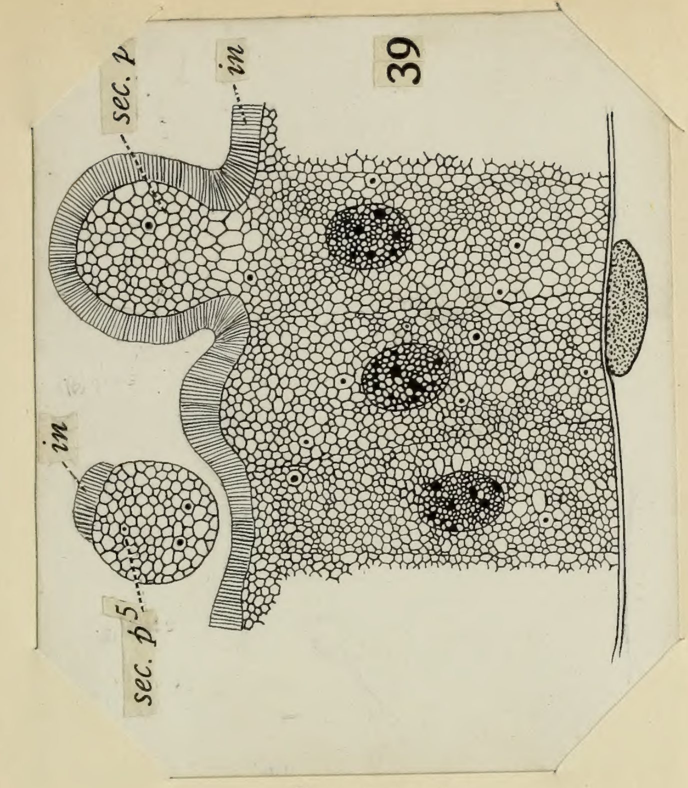

Fig. 28 (b).

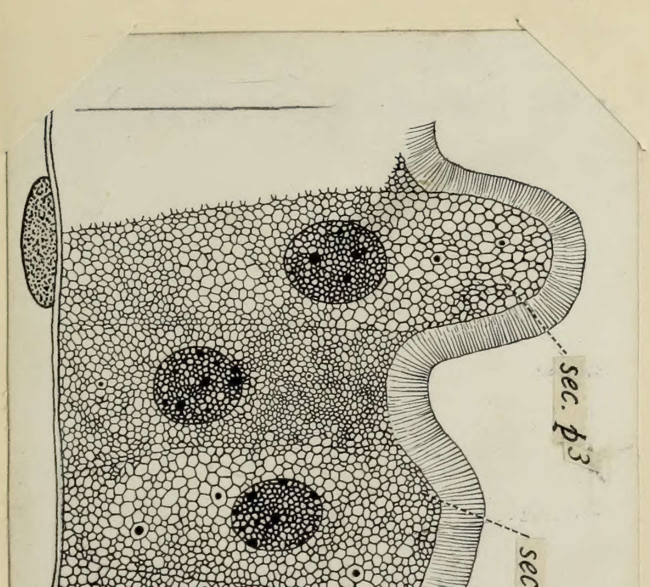

Fig. 28 (a).

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